

# The Transition between Immune and Disease States in a Cellular Automaton Model of Clonal Immune Response

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## Abstract

In this paper we extend the Celada-Seiden (CS) model of the humoral immune response to include infectious virus and cytotoxic T lymphocytes (cellular response). The response of the system to virus involves a competition between the ability of the virus to kill the host cells and the host's ability to eliminate the virus. We find two basins of attraction in the dynamics of this system, one is identified with disease and the other with the immune state. There is also an oscillating state that exists on the border of these two stable states. Fluctuations in the population of virus or antibody can end the oscillation and

drive the system into one of the stable states. The introduction of mechanisms of cross-regulation between the two responses can bias the system towards one of them. We also study a mean field model, based on coupled maps, to investigate virus-like infections. This simple model reproduces the attractors for average populations observed in the cellular automaton. All the dynamical behavior connected to spatial extension is lost, as is the oscillating feature. Thus the mean field approximation introduced with coupled maps destroys oscillations.

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## I. INTRODUCTION

The immune system is the body's defense against attacks from foreign substances (called *antigens*) such as parasites, bacteria and viruses. It has two different branches to fight against specific antigens: the *humoral response* (mediated by antibodies) and the *cellular response* (cell-mediated). In this paper we present a cellular automaton model of how these two parts of the immune system interact with each other during the response to antigenic stimulation.

Celada and Seiden [1,2] have proposed a model for the humoral response based on cellular automata. The aim of this model is not to simulate the immune response in all its features, which is clearly an impossible task. Although it is impossible to replace *in vivo* or *in vitro* experiments with computer simulations, computer simulations may be a useful tool in performing *in machina* experiments, which can then be used to design the more expensive and time consuming *in vivo* and *in vitro* experiments.

The organization of the paper is the follows: in Sec. II we give a short account of the biological background, for a more complete description of the immune system see [3]. In Sec. III we sketch the main features of the Celada-Seiden model for humoral response. In Sec. IV we discuss our extension of the CS model which introduces cellular response into the automaton model, and we present the results of our computer simulations. In Sec. V a system of *coupled maps* is proposed to describe the time evolution of populations in the immune system during an infection. In this section we show the results of analytical and numerical analysis of this model and discuss the similarities and the differences between this approach and the cellular automata.

## II. BIOLOGICAL FEATURES

The defense mechanisms used by the body against an attack from antigens are several, they include: physical barriers, phagocytic cells, different clones of particular white blood

cells (*lymphocytes*) and various blood-borne molecules (e.g., *antibodies*). Some of these mechanisms are present prior to exposure to antigens and their action is non-specific, i.e., they do not discriminate between different antigens and their response doesn't change upon further exposure to the same antigen. This kind of response is called *natural immunity*. There are other mechanisms with more specific behavior, they are induced by antigens and their response increases in magnitude and defense capabilities with successive exposures to the same antigens. These mechanisms are called *acquired* (or *specific*) *immunity* and are what we consider in this paper.

The main features of specific immunity are:

- *Specificity*: the immune response is highly specific for distinct antigens, that is only a small number of particular membrane receptors recognize a given antigen. These receptors bind specific portions of the antigen (called *antigenic determinants* or *epitopes*). Only a small fraction of the system recognizes any particular epitope, and cells which do recognize antigenic epitopes are positively selected by this recognition process so that their population increases (*clonal selection*).
- *Diversity*: the possible number of lymphocytes with different specificity (different *clones*) is extremely large ( $\geq 10^9$ ), although the total diversity actually existing in an animal is much smaller. The diversity is due to the variability of lymphocyte receptors, which derives from the mechanism of genetic recombination that occurs during cell development.
- *Maturation*: the response evolves by increasing the average affinity to the antigen through competition and selection for binding following mutation of the genes coding for the B-cell receptor.
- *Memory*: exposure of the immune system to an antigen enhances, in quality and quantity, its capability to respond to a second exposure to the same antigen (*secondary response*). This is due mainly to the persistence, after any response, of re-stimulable

cells (memory cells) ready to mount a new response to the same antigen.

- *Discrimination of self from non-self*: the immune system doesn't respond to substances produced by the human body (*tolerance to self*).

The specificity of the immune response is due to a class of white blood cells called lymphocytes. These are not able to begin a response without the help of various types of cells known as *antigen presenting cells* (APC), such as macrophages, dendritic cells, etc. Lymphocytes are present in the blood, lymph and lymph nodes; in the human body there are about  $10^{10}$  lymphocytes. The two major classes are: *B* and *T lymphocytes*.

In mammals B lymphocytes (so called because in birds they are produced in an organ called the *Bursa Fabricii*) mature in the bone marrow, they are able to bind antigen and to produce antibodies with the same specificity as their membrane receptors. Upon binding antigen a B cell will endocytose the antigen into small pieces between about 8 to 15 amino acids long (peptides). These pieces are bound to surface receptors called major histocompatibility molecules (MHC class II). Helper T cells are able to recognize and bind to these MHC/peptide complexes. When they do they trigger T-and B-cell proliferation and differentiation into memory cells and *plasma cells* (which produce antibodies).

T lymphocytes are born in the bone marrow, and then migrate to the thymus where they mature and are selected. Here a lot of T lymphocytes are destroyed to avoid autoimmune responses (*clonal deletion*). There are two different types of T lymphocytes which are important for our model:

- *helper T cells* (Th) which are involved in B-cell activation; they have receptors that can bind MHC/peptide complexes. As a consequence of this process, they produce a set of particular molecules, called *cytokines* (e.g., interleukin 2 and 4), that activate B and T cells;
- *cytotoxic T lymphocytes* (CTL) which can recognize, in an antigen specific way, cells infected by viruses, and kill them by lysis. The recognition is similar to that of helper T

cells in that pieces of antigen are presented on another type of MHC molecule (class I).

Specific immune responses are classified into two different types, based on the components of immune system that mediate the response.

- *Humoral response*, where B cells recognize antigens and produce antibodies that attack them. All these processes are mediated by Th cells. This is the main defense against extra-cellular microbes.
- *Cellular response*, mediated by CTLs which recognize infected cells and eliminate them (help is also needed from Th cells). This response is effective against intra-cellular viruses.

### III. A MODEL FOR THE HUMORAL RESPONSE.

A cellular automaton model implementing the humoral response (to be called CS herein) has been recently proposed [1]. A cellular automaton is a dynamical system with the following characteristics [4]:

- it has a discrete number of sites;
- it evolves in discrete time steps;
- each site can take a finite number of values;
- the evolution rule is deterministic;
- the evolution rule of a site depends only on a local neighborhood of sites around it.

The CS automaton modifies and extends these rules, allowing probabilistic evolution, making the evolution rules depend only on entities on the same site and permitting entities to move to neighboring sites. These are typical features of reactive lattice gases (see [5] for a review). The CS automaton is defined as a two dimensional lattice, usually of a small size ( $15 \times 15$ ), that represents a small part of the body.

### A. Components of the model system

An important characteristic of the CS model is the simulation of diversity in antigens and response by the introduction of several clonotypic elements (e.g., epitopes, peptides, and receptors) represented by binary strings. The objects present in the model are:

- cells (APC, B cells and T cells);
- antibodies;
- antigens.

B and T cells are represented by different clones, each clone is characterized by its surface receptor which is modeled by a binary string of  $N$  bits with a fixed directional reading frame. In Fig. 1a we show how these objects are represented in the model. Each clonotypic set of cells has a diversity of  $2^N$ ; for the simulations described in this paper we use  $N = 8$ , so we have a diversity of 256 (in comparison to  $\sim 10^9$  of the real system). The number of possible states in a site is therefore very high, for example if the maximum number of cells of each kind is 1000, the number of states in each site is  $\sim 10^{1536}$ .

Besides the receptor (BCR), B cells have MHC class II molecules on their surface. In the model they are also represented by a binary string of  $N$  bits. MHC's are involved in the process of B-cell activation. MHC diversity in a given body is very small, less than 10 different MHC molecules are present. We have generally used one or two different kinds of MHC molecules in the simulations.

We have used a complete repertoire for B cells, i.e., all can be produced by the system and, for the parameters we use here, the average occurrence of each clonotype in the starting population is approximately one. For T cells we begin with the complete repertoire, but they are filtered by the *thymus* before putting them in the lattice. The thymus is an organ through which T cells must pass before they can mature. In the thymus they are exposed to self-antigens presented on APCs. If they respond too strongly or not at all they are killed.

Therefore, the thymus acts as a filter to remove dangerous or non-responsive T cells. It is the body's first line of defense against autoimmune disease. Including a thymus, results in a T-cell population that has restricted diversity.

APCs represent non-specific antigen presenting cells (such as macrophages), they have no specific receptor (they can bind any type of antigen with a fixed probability) but they have the same MHC as B cells.

Antigens (Ag) are made of two different parts: *epitopes* and *peptides*. The epitope is the portion of an antigen that can be bound by the BCR; after this event the peptide (or better a peptide of the antigen, because generally an antigen has more than one peptide or epitope) is presented on an MHC molecule for T-cell cognate recognition (we show a sketch of the process in Fig. 1b).

Antibodies (Ab) are also made of two parts. They have a receptor (*paratope*), that is represented by the same string as the BCR of the parent B cell which produced them and, optionally, a peptide (*idiotope*).

## B. Interactions

There are precise interaction rules; the allowed interactions are mainly of two types:

- *specific*, as between antigen and antibody, antigen and BCR, or MHC-peptide complex and T cell receptor; this kind of interaction has a probability of interaction (affinity) evaluated according to the number of complementary bits between the binary strings that represent the receptors. In our simulations we have used a probability of 1 in case of perfect complementary, 0.05 for the case of one mismatching bit, and 0 for more than one mismatching bit.
- *non-specific* as APC-antigen interaction. The interaction takes place with a fixed clone-independent probability, typically equal to 0.002.



The main process of humoral response is B- and T-cell proliferation (clonal growth) and consequent antibody production. The process is divided into four parts: antigen-BCR interaction, endocytosis and peptide presentation on MHC, T cell recognition of the MHC/peptide complex, and finally, cell proliferation and differentiation into plasma cells (which produce antibodies) and memory cells (which provide the possibility of a further enhanced response). For each antigen several B-cell clones have receptors able to bind it. After binding, the B cell processes the antigen and presents it on its MHC and then waits for a recognition/binding event by a T cell. This is the signal for the B cells to proceed in the response. The signal may fail to be delivered either because the specific T cells have been negatively selected in the thymus, or because the probability of finding the right combination of B and T cells in the same site is low. The latter situation may change with time, e.g., after the T cells have proliferated through stimulation by APC presenting the same antigen. After they have received this second signal B cells can divide, producing memory and plasma cells. The plasma cells secrete antibodies, having the same receptor as the B cell, which can bind the antigen just as the BCR does. Upon T-B cell interaction the T cells also proliferate.

### **C. Simulations**

During a time step each site is considered individually. Each entity in a site is given an opportunity to take part in all interactions for which it is able. The success or failure of an interaction is determined by comparison of its probability with a random number. Although all possible interactions are considered, an entity can have at most one successful interaction on any one time step. After the interactions are determined, the entities are allowed to die (with some half life), stimulated cells divide, new cells are born, and antibodies are generated. Finally, the entities are given an opportunity to diffuse to neighboring sites. This constitutes a time step and the entire process is repeated for as many time steps as desired.

To give a rough idea of the functioning of the CS model we show a typical simulation of *immunization* in Fig. 2. The system initially has no antigen, no antibodies, and 1000 B cells, 1000 T cells and 1000 APCs uniformly distributed in space and receptor type (except that the T cells have been processed in the thymus); no interaction among species is present and the system is in a steady state where the natural death rate equals the birth rate. We start by injecting a single type of antigen (same epitopes and peptides). The antigens are at first bound primarily by the APCs since, although weakly binding, they are much more plentiful than the rare B cells that match the antigen. T cells will stimulate these APCs and then divide to form populous clones. There will then be sufficient antigen responding T cells to easily find the few responding B cells. Upon stimulation from the T cells the B cells also divide and form significant clones. Finally, antibodies are produced and the antigen is removed. This is the *primary response*.

If later more of the same antigen is injected (e.g., at time step 100 of Fig. 2) it is removed much more rapidly because the system has an appreciable population of B and T memory cells induced by the primary antigen dose. This is the *secondary response* and it can be so strong and swift that the antigen is eliminated before it can do any damage.

A number of other aspects of the humoral immune system have been studied with this model, e.g., response to various levels of antigen dose [1], hypermutation and affinity maturation [6], and thymus function [7].

#### IV. INFECTION AND CTL RESPONSE.

We have introduced three new features into the CS model:

- *infection*
- *CTL response*
- *cross regulation*

The aim is to implement some mechanisms of cross regulation and study how the cellular and humoral responses interact with each other. The CTL response is activated against intracellular viruses or parasites, so before introducing CTL we need an infection step. Most of our simulations are limited to understanding the features of infection.

In Fig. 3 we sketch the processes modelled in CS model (in bold) and the modifications we have added to implement CTL response (in italics).

### A. Infection

We consider one kind of antigen, a virus with one epitope and with infective function, i.e., capability to penetrate cells and to multiply inside them, like a virus or an intracellular parasite. All references to antigen from now on refer to this virus. It has a given probability  $P_i$  per time step to infect any B cell or APC present in the same site. The target cells are the cells of immune system: B cells and APC. We chose to infect only these to limit the cell species in the model but infections of this type are known, for example, *Epstein-Barr* virus which infects B cells and *Leishmania major* an intracellular parasite of macrophages.

Infected cells continue their *normal* life while the virus duplicates inside them with a constant growth rate ( $r$ ) (this may be a drastic approximation of realistic situations). That is the number of viruses inside the cell at time  $n$  ( $V_I^n$ ) grows according to:

$$V_I^{n+1} = r \times V_I^n$$

When the number of viruses inside the cell exceeds a fixed threshold ( $V_{\max}$ ), the cell is destroyed and  $V_{\max}$  viruses are freed. The virus is shielded from antibodies when it is inside the cell but can be destroyed when it is outside. In our first simulations, featuring only humoral response, virus inside the cells is safe; later we will consider the case where lymphocytes (CTL) can recognize and kill infected cells.

A first set of simulations was performed by introducing a single injection of virus and then observing the response of the system as a function of  $V_{\max}$  and  $P_i$ .

We have observed three different final states:

- indefinite growth of the virus (**V**, diseased state), see Fig. 4 (right-hand panels);
- elimination of the virus (**IS**, immune state), see Fig. 4 (left-hand panels);
- an oscillatory state (**O**), see Fig. 4 (middle panels).

Fig. 4 presents the total number of B cells and virus versus time. In Tables I and II we summarize the results of the simulations. There are two fixed point type basins of attraction **IS** and **V**. The **O** state appears at the border between these two.

TABLE I. Final state for various values of  $P_i$  and  $V_{\max}$

	$P_i = 0.1$	$P_i = 0.05$	$P_i = 0.025$	$P_i = 0.01$
$V_{\max} = 8$	<b>V</b>	<b>IS</b>	<b>IS</b>	<b>IS</b>
$V_{\max} = 12$	<b>V</b>	<b>O</b>	<b>IS</b>	<b>IS</b>
$V_{\max} = 16$	<b>V</b>	<b>V</b>	<b>V</b>	<b>IS</b>
$V_{\max} = 25$	<b>V</b>	<b>V</b>	<b>V</b>	<b>O</b>
$V_{\max} = 32$	<b>V</b>	<b>V</b>	<b>V</b>	<b>V</b>

TABLE II. Final state for  $P_i = 0.05$

$V_{\max}$	6	7	8	9	10	11	12	13	14	16	32
Final state	<b>IS</b>	<b>IS</b>	<b>IS</b>	<b>O</b>	<b>O</b>	<b>O</b>	<b>O</b>	<b>V</b>	<b>V</b>	<b>V</b>	<b>V</b>

If we look at the antigen concentration in the **O** case we see low numbers of B cells correspond to high values of virus, i.e., they are in phase opposition. The population of virus is extinguished when the concentrations of free and intracellular virus are so low that they cannot infect enough cells before being eliminated by antibodies. So at low concentration of antigen, it may happen that there is not enough at some time to restart the process of infection; this leads to the elimination of antigen and to the recovery of the initial concentration of B cells (**IS**). However the opposite process is also possible: in the high-antigen

concentration phase, virus can kill most of the clones that are able to initiate the immune response. So after a number of oscillations, it is possible to find that the system suddenly relaxes to the diseased state ( $\mathbf{V}$ ). Fluctuations in the number of virus in the former case, or in the number of B cells in the latter case, can destroy the oscillating mode.

The period of oscillation is much larger than the characteristic time of infection and the latency period (i.e., the time in which virus grows inside an infected cell). In fact, in our simulations, the latency period is about 10 time steps while the oscillation period is, at least, ten times larger.

The occurrence of these global oscillations depends on the diffusion of cells, antibodies and virus throughout the whole body array. Increasing the diffusion constant causes the oscillations to become more regular, fluctuations in time delay between high virus concentration phases are smaller, i.e., frequency modulation decreases. For low values of the diffusion constant the system is less synchronized and at a certain threshold value global oscillations disappear.

At the beginning of the cycle virus quickly infects cells and starts growing. At this time if the B cells can produce enough antibodies, before being killed by virus, some *islands* free of virus grow randomly. (A similar phenomenology has been observed in a very different framework in studying a cellular automaton model for catalytic oxidation of  $CO$  on a  $Pt(100)$  surface [8].) The regions free of virus become larger, the islands merge together and, after some time, they cover the whole lattice. This is the low infection phase characterized by low dose of virus and by the presence of large numbers of antibody over the whole lattice. Antibodies have a natural time decay, if some virus survives the high antibody concentration, for example if they find a host cell to infect before antibodies can eliminate them, they can trigger the infection process again after most of the antibodies are removed by their natural decay.

We carried out simulations to verify this mechanism by varying the antibody decay time. We found that for low values of the antibody lifetime the high-dose antibody phase is much shorter. For a lifetime of one time step, oscillations disappear and we observe a steady state

presence of virus.

## B. The CTL response

The next step for implementation of the cellular response in the model is the introduction of another population of cells: *cytotoxic T lymphocytes* (CTL). In our model CTL are not specific, they have a fixed probability  $P_K$  to interact, and to kill, infected cells. In nature the CTL response is specific, however we can think of our single CTL as equivalent to working with just one kind of antigen, i.e., the CTLs we introduce are precisely those which interact with that antigen.

In reality CTLs interact with infected cells by recognizing antigenic peptides presented by class I MHC molecules. The result is the destruction of the infected cell and the clonal expansion of the CTLs. At the same time B cells, helped by lymphocytes, produce antibodies that can kill or inactivate free virus. Thus, the cellular and humoral responses act in parallel against different viral targets. It is not known whether the two responses are synchronized but they do have a similar degree of complexity in their activation and effector steps. In our simulation the CTL process is simplified and short, since it consists of a single step.

We have performed a series of simulations to evaluate the effect of CTLs by varying their killing capability  $P_K$ . Furthermore, we assess how the antibody and CTL responses cooperate. Analyzing results of the simulations we observe that, of course, the presence of CTLs helps the immune response. In some cases CTLs eliminate infection without the help of antibodies (or use antibodies only in the last part of the response), and sometimes cooperation of antibodies and CTL is needed.

We have tried to perturb the **O** state of the system without CTL's by introducing a small number of CTL, as expected we observe that oscillations are still present for low values of CTL, while they are eliminated for large amounts of CTL.

We ran another series of simulations introducing a cross-regulation mechanism between CTL and B-cell activation. In the real system these processes are mediated by two different

sets of cytokines involving various *interleukins* (IL) and *gamma interferon* (IFN- $\gamma$ ). We have modelled this mechanism by introducing two different model cytokines called IL and IFN. The former is produced by B-T cell binding and down-regulates CTL division, while the latter accompanies CTL activation and down-regulates B-cell division (in the human body these processes exist, but are much more complex and involve many different proteins). In the presence of this cross-regulation mechanism, the system quickly chooses one type of response, cooperation is weaker and in only a few simulations have we observed that both responses are activated at different times.

We have also found an oscillatory state here, but oscillations fade to an intermediate state between **V** and **IS**; this is a low infection state where virus is always present in small doses as in a chronic disease.

We also did simulations showing that we can drive the choice of the system by putting an initial amount of IL or IFN in the lattice at  $t = 0$ ; then one path is enhanced at the beginning which inhibits the other.

### C. Comparison to biological data

Upon introducing an infection mechanism, even without CTLs, we obtain two fixed points: one characterized by indefinite virus growth (**V**), the other by elimination of the virus (**IS**); these states can be identified with *disease* and with *recovery* from a viral infection.

In the case of evolution to **V**, our simulations show a condition of *immunodeficiency*, i.e., a sudden reduction of the number of cells of the immune system. We haven't found cases of this sort of behavior for B cells, but there are a lot of data for a T helper cells in AIDS (*acquired immunodeficiency syndrome*). The mechanism is different because HIV (the virus of AIDS) infects and kills T cells.

In our model we also have an oscillating mode **O**. Oscillatory immune responses are found in various experiments *in vivo* and *in vitro* [9–11]. Most of these are devoted to study immune response to a non-proleferating antigen, such as LPS (lipopolysaccharide, a sugar molecule)

[9,10] or bacterial levan [14]. Cyclic response of T and B cells are found. The feedback mechanisms involved in the generation of a cyclic response are generally unknown, however three different feedback mechanism are proposed to explain these oscillatory patterns. The first one is an antibody feedback mechanism: after the first antigen injection, B cells produce specific antibodies that bind the antigens and form antibody-antigen complexes which block further antigenic stimulation and antibody production. If the antibody concentration becomes too low the complexes may dissociate before being eliminated by catabolism so that the antigens become free and a new cycle can start. A time delay differential equation model of this process was proposed by Grossman *et al.* [12]. Other feedback mechanisms that have been proposed, are the presence of auto-anti-idiotypic antibodies according to Jerne's network hypothesis and the regulatory effects of T helper cells [13,14].

In our system the feedback mechanism is strictly connected to viral growth, so it is quite different from those previously mentioned, although the need for regulatory T cells for generating the oscillations and the presence of high and low antibody concentration phases are similar in both cases. In fact in such cases, antigens (i.e., LPS, bacterial levan) cannot proliferate after the first injection, while in our case we deal with an infection due to a microorganism (virus or parasite) that can grow.

Closer to our case are oscillations found in some infectious diseases. Lo, Wear, *et al.* [15] have studied an infection due to *Mycoplasma fermentans* isolated from Kaposi's sarcoma of a patient with AIDS. They inoculated silver leaf monkeys with this antigen and found that all infected animals exhibit oscillations in antigen concentration in the blood. Another kind of infectious disease where an oscillatory pattern can be found is malaria. Malaria is mainly due to two parasites *Plasmodium vivax* and *Plasmodium falciparum* and it presents periodic sharp episodes of high fever (*paroxysms*). The cyclic behavior of the disease can be monitored by measuring fever or the concentration of cytokines involved in the response to the malarial infection [16].

In presence of CTL and of a simple model of regulation our system can choose between two different paths: humoral or cell-mediated response. *In vivo* this choice is apparently due



to the presence of different patterns of cytokines (see [17]) which arise from two subclasses of T-helper cells called Th1 and Th2. Although they are all T-helper cells there is a subtle differentiation between those that help humoral (Th2) and cellular (Th1) responses.

A model for Th1-Th2 cross-regulation has been proposed by Fishman and Perelson [18] based on a system of ordinary differential equations. The regulatory mechanism between Th1 and Th2 is implemented by introducing two species of cytokines. The model shows that the immune response is due mainly to one subset of T helper cells, *i.e.*, they haven't found any stable fixed points characterized by the contemporaneous (valuable) presence of both kinds of T helper cells. The relative efficiency of activation of the responding Th1 and Th2 cells is the crucial parameter that drives the choice of the system for one kind of response. We have also obtained a similar result in our model by changing the capability of CTL and B cells to be inhibited by the presence of cytokines. We don't have Th1 and Th2 cells in our model, but we have introduced these two sets of cytokines with regulatory function (called IFN and IL in the model) as a very preliminary step towards Th1-Th2 system. Therefore, as we have seen in Section IV B, we obtain similar cross-regulation.

Fishman and Perelson also studied the evolution of the system by varying the initial dose of antigen. They found that, in a particular region of parameter space, different doses of antigen can induce a different kind of response. We haven't found any such behavior in our model.

Another differential equation model of Th1-Th2 regulation has been proposed by B. Morel, *et al.* [19]. This model gives a detailed description of the process of interactions between Th1 and Th2, involving different kinds of cytokines and phases of maturation of T cells. In particular they have shown applicability of this kind of model to immunological experiments *in vitro* and the possibility of using these experiments for a parametric estimation of the model. However, they have not yet used their model to simulate an antigenic attack, so comparison with our results is not possible.

## V. COUPLED MAP MODEL

We have built a simple system of coupled maps to model the infection process. We have preferred to use a coupled map model with respect to a system of differential equations to preserve the time discreteness of the cellular automaton model. There are, of course, important differences between discrete and continuous time dynamics; coupled maps reproduce reasonably well the results obtained in the cellular automaton model in the considered range of parameter values and are easier and faster to simulate, although, as we will see in our case, they do not reproduce the oscillatory state.

We consider four different species:

- B cells, a certain clone of B cells which is able to interact with the injected antigen, we call this population  $B$ ;
- infected B cells, we do not consider the possibility that a B cell can be infected by more than one virus;
- free virus,  $Ag$ ;
- active B cells, those B cells who have recognized the antigen. B cells need a second signal from T cells to be activated, this is modeled by an activation function  $T$ . Infected B cells and active ones are not available for activation.

We have developed a mean-field model for the kinetics of the species, considering only averaged concentrations and neglecting diffusion. The equations that describe the population dynamics are:

$$\begin{aligned}
 B_{n+1} &= B_n + s - \mu B_n - \delta B i_n + d\sigma B a_n \\
 B i_{n+1} &= B i_n - (\delta + \mu) B i_n + P i_n (B_n - B i_n) \\
 B a_{n+1} &= B a_n + P a_n (B_n - (B i_n + B a_n)) - \lambda B a_n \\
 A g_{n+1} &= A g_n + r\delta B i_n - P i_n B_n - a(1 - \sigma) B a_n + \\
 &\quad - P a_n (B_n - (B i_n + B a_n))
 \end{aligned} \tag{5.1}$$

with:

$$\begin{aligned} P i_n &= 1 - (1 - \alpha)^{A g_n} \\ P a_n &= 1 - (1 - \beta T_n)^{A g_n} \\ T_n &= \epsilon + (1 - \exp(-\lambda B a_n)); \end{aligned}$$

$n$  is the time step index and species concentrations are positive real numbers.

In the first equation of the system (5.1)  $s$  is the source term due to bone marrow production and  $\mu B_n$  is the exponential death term,  $\mu$  is the reciprocal of the average lifetime.  $\delta B i_n$  is the number of infected B cells that die as a consequence of infection.  $d\sigma B a_n$  is the clonal expansion term, i.e., the B cells that divide after being activated:  $d$  is the number of B cells produced per time step by an activated B cell ( $d = 1$  if the time step is the division time) and  $\sigma$  is the fraction of active B cells that divide at each time step, thus  $1 - \sigma$  is the fraction of activated B cells that become plasma cells and secrete antibodies.

The second equation describes the time evolution of the number of viruses inside a cell  $B i$ .  $(\delta + \mu) B i_n$  is the death term and  $P i_n (B_n - B_i)$  the infection term. The probability  $P i_n$  that a cell is infected by at least one virus is computed as in the cellular automaton simulation. We have assumed that the mean free time between two cellular and molecular collisions is small compared to the map time step which is thought to represent the division time of B cells. Infection processes are assumed independent, so let  $\alpha$  be the infection probability,  $1 - \alpha$  is the probability of not being infected by a given antigen,  $(1 - \alpha)^{A g_n}$  is the probability of not being infected by any antigen, and  $1 - (1 - \alpha)^{A g_n}$  is the probability of being infected by at least one antigen per B cell and  $(1 - (1 - \alpha)^{A g_n})(B_n - B_i)$  is the total number of infected B cells per time step, because only uninfected B cells are available for infection.

The third equation describes the evolution of active B cells.  $P a_n (B_n - (B i_n + B a_n))$  is the growth term due to B cell activation; the probability for a B cell to be activated by at least one antigen ( $P a_n$ ) is computed in the same manner as  $P i_n$ , but the probability of activation is  $\beta$  times a certain activation function ( $T_n$ ) that represents the effect of T cells.  $T_n$  introduces a positive feedback. At the beginning of the response the number of active T

cells is small; then, because of B cell-T cell binding followed by B-cell activation and T-cell duplication, the effect becomes larger. To represent this we have chosen the function:

$$T_n = \epsilon + (1 - \exp(-\lambda Ba_n));$$

with  $\epsilon$  the minimal activation probability in absence of active B cells. We consider only those B cells that are not infected or yet activated to be available for activation. The last term of the equation,  $-\lambda Ba_n$ , is the inactivation term, where  $\lambda$  is the reciprocal of the average activation lifetime. We neglect the possibility that active B cells die ( $\lambda \gg \mu$ ).

The last equation of the system (5.1) is the evolution equation for free antigens, the source term is due to death of infected B cells, each of them releasing  $r$  antigens. Some antigens enter the cells because of the infection process ( $-Pi_n(B_n - B_i)$ ) and of the internalization following B-cell activation ( $-Pa_n(B_n - (Bi_n + Ba_n))$ ). Each plasma B cell produces  $a$  antibodies, that eliminate  $a(1 - \sigma)Ba_n$  antigens.

We have to impose some more constraints on (5.1), because, for example,  $Pi_n(B_n - B_i)$ , the number of B cells that are infected, has to be smaller than both  $B_n - B_i$  (and this is always verified) and  $Ag_n$ , this last condition is implemented by the *min* function. The same argument is true for other terms. After having imposed these constraints we obtain the system:

$$\begin{aligned} B_{n+1} &= B_n + s - \mu B_n - \min(\delta Bi_n, B_n) + d\sigma Ba_n \\ Bi_{n+1} &= Bi_n - \delta Bi_n + \min(Pi_n B_n - B_i, Ag_n) \\ Ba_{n+1} &= Ba_{n+1} + \min(Pa_n(B_n - (Bi_n + Ba_n)), Ag_n) - \lambda Ba_n \\ Ag_{n+1} &= Ag_n + r\delta Bi_n - \min(Pi_n B_n, Ag_n) - a(1 - \sigma)Ba_n + \\ &\quad - \max(\min(Pa_n(B_n - (Bi_n + Ba_n)), Ag_n), 0) \end{aligned} \tag{5.2}$$

## A. Numerical results

We have studied the response to an antigenic stimulation. Thus our typical initial condition in the  $(B, Bi, Ba, Ag)$  space is  $(B_o, 0, 0, Ag_o)$ . We carried out simulations varying

$r$  and  $\alpha$ , the parameters corresponding to  $P_I$  and  $V_{\max}$  in the cellular automaton model. Extensive simulations show that in all these cases the system evolves to one of these two fixed points:

- $(\frac{s}{\mu}, 0, 0, 0)$  with antigen elimination which we call the immune state ( $IS$ );
- $(\frac{s}{\mu+1}, \frac{s}{(\delta+\mu)(\mu+1)}, 0, +\infty)$  with antigen growth which we call the diseased state ( $V$ ).

$IS$  is reached for small values of  $r$  and  $\alpha$ , otherwise the system evolves to  $V$ . We have verified numerically that the system (5.2) has other fixed points but they are all locally unstable, also  $IS$  is locally unstable in some range of values of  $\alpha$  and  $r$ .  $IS$  lies on the border of the domain of definitions of our dynamic variables (i.e. positive real numbers), so we have studied its stability considering only allowed (i.e. positive) perturbations.

The mechanism by which  $IS$  is reached is inherently linked to the time discreteness of the dynamics and to the fact that the stable manifold of  $IS$ ,  $(B, 0, 0, 0)$ , belongs to the boundary of the domain. The rule by which we update the values of concentrations when they become negative is to set them to zero, which is a boundary value, and it may then be attracted to  $IS$  if it happens to be close to the stable manifold. This kind of mechanism represents closely what happens in the cellular automaton model, where the discreteness of the state variable may lead to the disappearance of a species in one time step by fluctuations.

We have fixed a value for  $\alpha$  ( $\alpha = 0.05$ ) and studied the modifications of the basin of attraction of  $IS$  with initial conditions of the class  $(B_o, 0, 0, Ag_o)$  (*antigenic stimulation*), varying  $r$ .

We have observed that  $IS$  is inside its basin for low values of  $r$ , so  $IS$  is stable for  $(\frac{s}{\mu} + \epsilon, 0, 0, \epsilon')$  like perturbations, with  $\epsilon, \epsilon' \ll \frac{s}{\mu}$ . Vice versa it is at the border for  $r > r_o$  ( $r_o \simeq 11.045$  with  $\alpha = 0.05$ ) and  $(\frac{s}{\mu} + \epsilon, 0, 0, \epsilon')$  belongs to the basin of attraction of  $V$ . In Fig. 5 we plot the basins of attraction of  $IS$  and  $V$  in the  $(B, 0, 0, Ag)$  plane under *antigenic stimulation*; the border between the two basins moves with  $r$ . We observe that the border curve in the  $(B, Ag)$  plane is not monotonic with  $B$ . Analysing the temporal behaviour of the

four populations for various initial  $B$  values, with other parameters fixed, we find the ratio  $\frac{Bi}{Ba}$  to be the crucial parameter for the choice between evolution to one fixed point or the other. In fact, in the  $IS$  case, after an initial transient,  $\frac{Bi}{Ba}$  is below 1, while in the  $V$  case it is greater than one. Both populations,  $Ba$  and  $Bi$ , increase with the number of initial  $B$  cells, but their ratio  $\frac{Bi}{Ba}$  after the initial transient is not monotonic in the initial value of  $B$ . This causes the dip in the curve of Fig. 5.

The loss of stability of  $IS$  is due to the fact that for  $r < r_o$  an orbit started close to  $IS$  returns near the stable manifold of  $IS$ , while for  $r > r_o$  the orbit goes away to  $V$ . In fact, if we plot the projections of some orbits, starting near  $IS$ , on the  $(B, Ag)$  plane we can see (Fig. 6) that close to the fixed point orbits with different values of  $r$  are similar but then, for  $r > r_o$ , the orbits evolve to  $V$ , while for  $r < r_o$  we have a sort of homoclinic phenomenon because the orbit reaches the stable manifold of  $IS$  (the  $B$  axis) and so  $IS$  itself. The  $\omega$ -limit set (see Ref. [20] pag.235) of an  $IS$  neighborhood is  $IS$  itself for  $r < r_o$ ,  $IS \cup V$  for  $r = r_o$  and  $V$  in the case of  $r > r_o$  (except the  $B$  axis always belongs to the stable manifold of  $IS$ ). We are presumably in the presence of a *global bifurcation* since the transition  $IS \Rightarrow V$  is not due to the loss of local stability but to the behavior of the orbits far from the fixed point.

It is also interesting to observe that the dynamics of the relevant species show a similar behavior to that found in the cellular automaton simulation. In Fig. 7 we show the time evolution of  $B$  cells and  $Ag$  concentrations in both the  $IS$  and the  $V$  case.

Therefore, using a simple mean field model we have found some features of the cellular automaton simulation, i.e.:

- evolution to  $IS$  or  $V$ ;
- same behavior when varying the parameters corresponding to  $P_i$  and  $V_{\max}$ ;
- similar behavior of populations in the  $IS$  and  $V$  cases.

We have not found oscillatory states (cycles or limit cycles) at the border between  $IS$  and  $V$ . Since in Section IV diffusion is shown to play an essential role in establishing oscillations,

we presume that spatial effects are relevant and could be added to the mean field model in a sort of diffusively coupled map lattice.

## VI. CONCLUSIONS

We have extended the Celada-Seiden (CS) model to include cellular response and we have studied the dynamics of an infectious disease and the cross-regulation between humoral and cellular response. With respect to the CS model we have included cytotoxic T lymphocytes (killer cells) and some generalized cytokines; moreover, the infecting virus is recognized by the immune system as antigen and is attacked both by antibodies outside an infected cell and by killer cells inside the infected cell. To our knowledge this is a first attempt to simultaneously treat the two main branches of specific immune response. Although a number of simplifications were made (e.g., non-specificity of T killer cells), some interesting results were obtained.

We performed a number of simulations and compared our results with *in vivo* and *in vitro* experiments. By varying the probability of viral infection and the number of viral particles released after cell lysis we obtained two fixed points towards which the systems evolve. These are identified with disease or recovery after the immune response. At the border between these two states we find persistent synchronized oscillations of all populations. This result is so robust as to be also present in the absence of killer cells. The introduction of killer cells obviously improves the immune response and, through cross-regulation mechanisms, biases the response towards one of the two branches, as is observed *in vivo*. We have also found that fluctuations can destroy the oscillating mode and drive the system to one of the stable fixed points.

We also discussed a mean field model based on coupled maps to study an infection due to a virus-like agent, with the aim of understanding the main feature of the CS simulations, i.e., evolution towards disease or recovery. This very simple model finds the same fixed points, and the temporal behavior of the concentrations of the pertinent entities are also

in agreement with the CS model. However, the dynamical behavior connected with spatial extension is lost, as is the oscillating mode. The mean field approximation introduced with coupled maps destroys the oscillations. This shows that the spatial effects introduced by the cellular automaton model are crucial to obtain the oscillatory state, which is quite an important and interesting new feature. The mean field limit behavior of the coupled map model can be reached by increasing the diffusion constant of CA model.

Our model is just a first attempt towards a complete implementation of cellular response in which both responses are simulated in specific manner, as in the real system. However the results presented in this paper show the wide possibilities of a microscopic approach to the study of immune response.

## **VII. ACKNOWLEDGMENTS**

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FIG. 1. a) Entities in the Celada-Seiden model simulation for the case of 8 bits. b) Process of recognition, internalization and presentation of antigen. Numbers indicate decimal values of the binary strings.

FIG. 2. A typical immunization experiment.

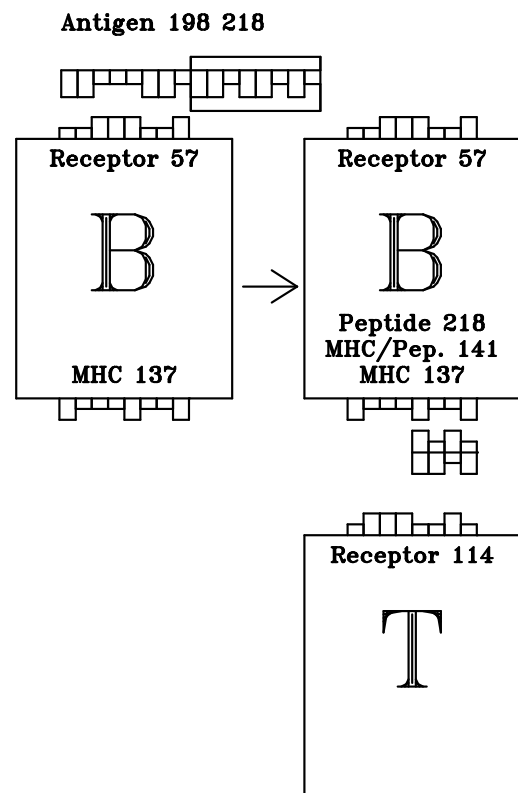
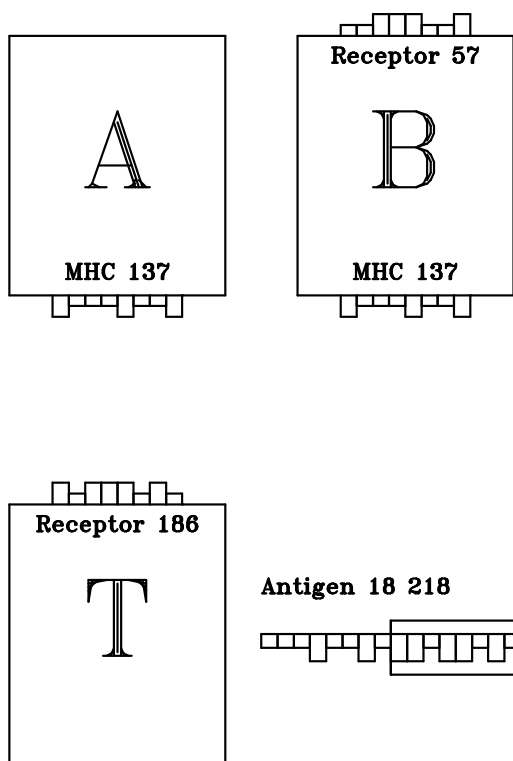
FIG. 3. A scheme of parallel humoral and CTL response with effectors and regulators. Bold type indicates the part of the response simulated by the basic Celada-Seiden model, italics the part simulated in the extended model.

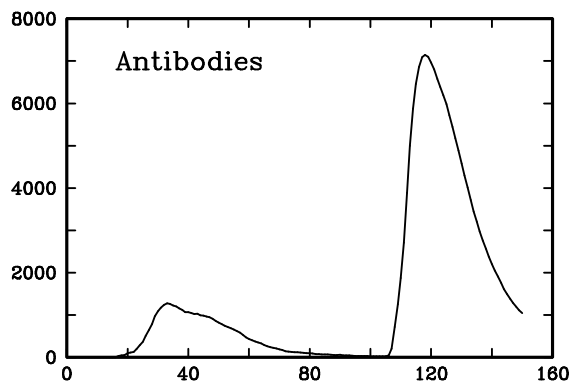
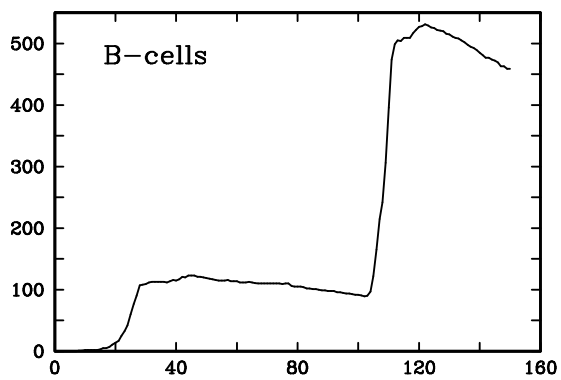
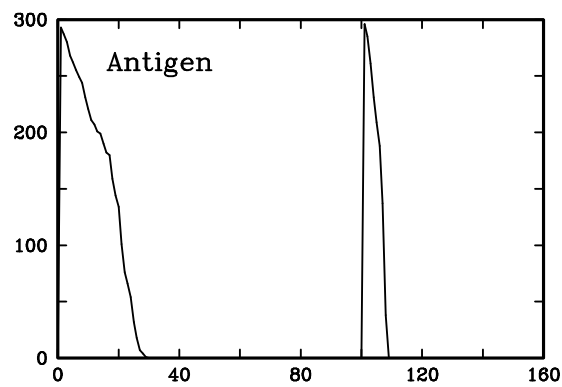
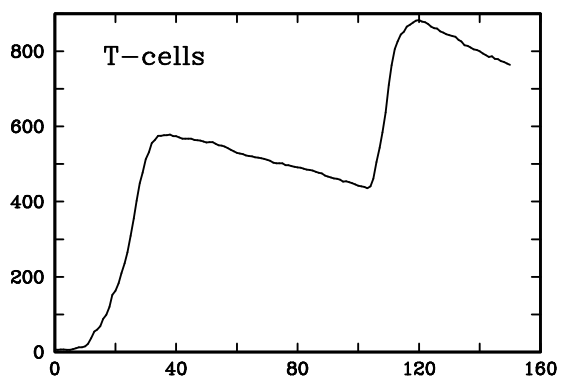
FIG. 4. Evolution of the population of B cells and antigens for three cases of  $V_{\max}$  with  $P_i = 0.05$ .

FIG. 5. The separatrix of the basins of attraction of  $IS$  and  $V$  for three different values of the number of viruses injected back into the system by virus induced cell death ( $r$ ). The lower right is the basin of  $IS$ , the upper left is the basin of  $V$ .

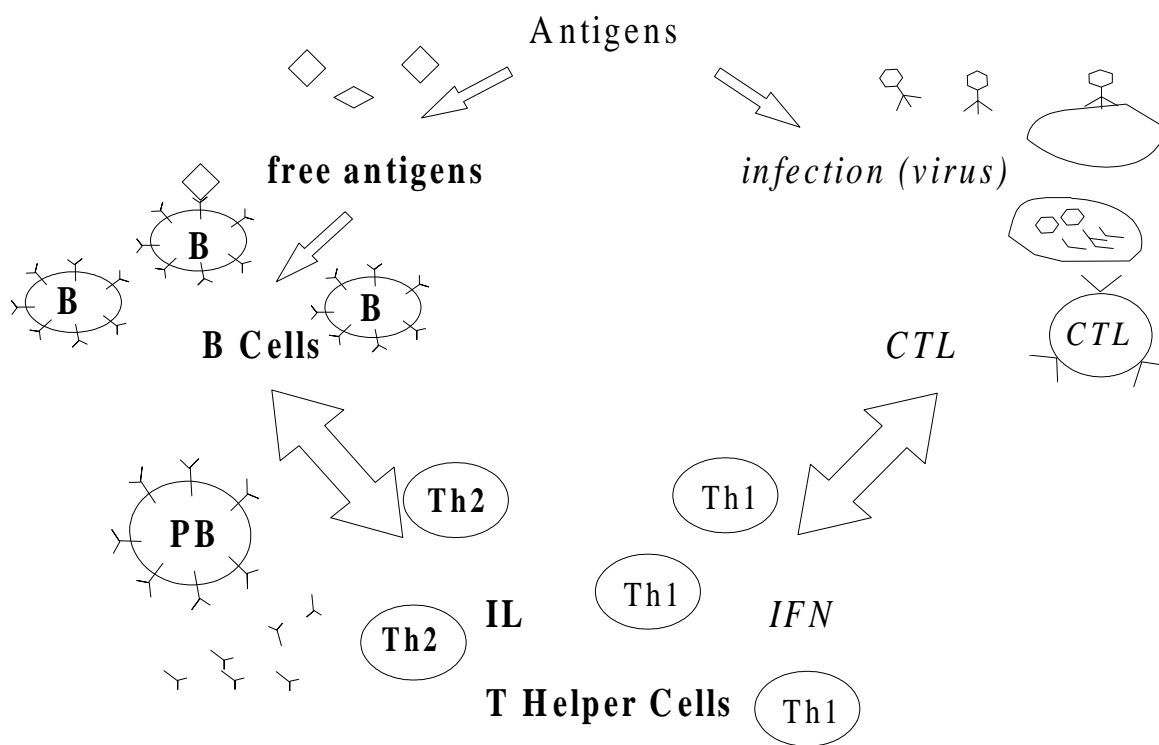
FIG. 6. Projections on  $(B, Ag)$  plane of some orbits for different values of  $r$  and same initial condition  $(\frac{s}{\mu}, 0, 10^{-3}, 0)$ .

FIG. 7. Evolution of concentration of B cells and antigens for the cases of  $IS$  and  $V$ .





Time Steps



**Figure 3**

